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cGMP signalling

Roelofs, Jeroen; Smith, Janet L.; Haastert, Peter J.M. van

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translated, but where the entire primary sequence of the protein is not the information that is under selection. In many cases, the number of amino acids under selection could be few or none, which can lead to the appearance of a high K_a/K_s ratio, even though there is selection for expression of the small protein sequence. For example, leader peptides are small proteins that have critical roles in gene expression through translational control [2]. Here, pausing of the ribosome during translation of a leader protein may allow for the formation of an anti-terminator RNA structure, thereby allowing transcription of the downstream genes in an operon. If the ribosome does not pause, a rho-independent terminator will form and the operon is not expressed. Leader peptides in the *Escherichia coli* genome control the *thr*, *leu*, *trp* and *phe* operons, and their K_a/K_s ratios are far greater than expected for protein-coding genes (three of them are even greater than Ochman's conservative $\mu + 2\sigma$ threshold, Fig. 1a), which would seem to indicate that these small ORFs do not encode proteins. In reality, only amino acids responsible for ribosome pausing when a charged tRNA becomes depleted (e.g. two tryptophan codons in the *trpL* gene) might be under selection for amino acid identity. The additional residues of the leader peptide are not under selection for the function of the protein (although they can participate in mRNA secondary structure formation). Similar leader peptides are found upstream of the *tnaA* tryptophanase gene [3,4] and chloramphenicol resistance genes [5] and operate by different methods. Similar small upstream ORFs occur in eukaryotic genomes (e.g. the 25-codon arginine-sensing peptide cotranscribed with the *Saccharomyces cerevisiae* CPA1 gene [6]). But the end result is the same: the short length, poor conservation and often unusual composition of these leader peptides can easily lead one to dismiss the coding potential of their genes.

In some cases, the majority of a peptide could be disposable, also leading to high K_a/K_s ratios. Although small disposable portions are commonly seen among leader sequences for secreted proteins, or pro-proteins made in inactive states (e.g. *Bacillus* pro- σ factors); the most extreme case might be the *pqqA* peptide, which is over-produced relative to other proteins in the *Klebsiella pqq*

operon and may serve as the substrate for the synthesis of the cofactor PQQ [7,8]. Here, the amino acids glutamate and tyrosine may be cleaved from the peptide backbone and serve as the substrate for cofactor biosynthesis, and the remaining residues may serve as a scaffold. Some transcribed regions may not encode polypeptides at all. The K_a/K_s test is useless in the identification of small functional RNAs, which can have important roles in cellular metabolism, and may be great in number [9]. Some of the regions designated as small ORFs – even those with genetic evidence for their importance – may act through an RNA product.

The manifold ways small protein products affect cellular metabolism make their identification onerous, and sometimes even comparisons with closely related genomes cannot aid in their unambiguous identification. In these cases, hands-on experimentation could be the only route towards gene discovery and the potentially fascinating insights in molecular biology that can result.

References

- Ochman, H. (2002) Distinguishing the ORFs from the ELF's: short bacterial genes and the annotation of genomes. *Trends Genet.* 18, 335–337
- Yanofsky, C. (1988) Transcription attenuation. *J. Biol. Chem.* 263, 609–612
- Stewart, V. and Yanofsky, C. (1986) Role of leader peptide synthesis in tryptophanase operon expression in *Escherichia coli* K-12. *J. Bacteriol.* 167, 383–386
- Gong, F. and Yanofsky, C. (2002) Analysis of tryptophanase operon expression *in vitro*: accumulation of TnaC-peptidyl-tRNA in a release factor 2-depleted S-30 extract prevents Rho factor action, simulating induction. *J. Biol. Chem.* 277, 17095–17100
- Lovett, P.S. and Rogers, E.J. (1996) Ribosome regulation by the nascent peptide. *Microbiol. Rev.* 60, 366–385
- Pierard, A. and Schroter, B. (1978) Structure–function relationships in the arginine pathway carbamoylphosphate synthase of *Saccharomyces cerevisiae*. *J. Bacteriol.* 134, 167–176
- Velterop, J.S. *et al.* (1995) Synthesis of pyrroloquinoline quinone *in vivo* and *in vitro* and detection of an intermediate in the biosynthetic pathway. *J. Bacteriol.* 177, 5088–5098
- Meulenberg, J.J.M. *et al.* (1992) Nucleotide sequence and structure of the *Klebsiella pneumoniae* *pqq* operon. *Mol. Gen. Genet.* 232, 284–294
- Wassarman, K.M. *et al.* (2001) Identification of novel small RNAs using comparative genomics and microarrays. *Genes Dev.* 15, 1637–1651

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cGMP signalling: different ways to create a pathway

Jeroen Roelofs¹, Janet L. Smith² and Peter J.M. Van Haastert³

¹Department of Cell Biology, Harvard Medical School, 240 Longwood Avenue, Boston, Massachusetts 02115-5730, USA

²Boston Biomedical Research Institute, 64 Grove Street, Watertown, Massachusetts 02472-2829, USA

³Dept of Biochemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

Recently, a novel cGMP signalling cascade was uncovered in *Dictyostelium*, a eukaryote that diverged from the lineage leading to metazoa after plants and before yeast. In

both *Dictyostelium* and metazoa, the ancient cAMP-binding (cNB) motif of bacterial CAP has been modified and assembled with other domains into cGMP-target proteins. The domain structures of these cGMP targets, as well as the enzymes responsible for cGMP synthesis and degradation, are entirely different between *Dictyostelium*

Corresponding author: Peter J.M. Van Haastert
(P.J.M.van.Haastert@chem.rug.nl).

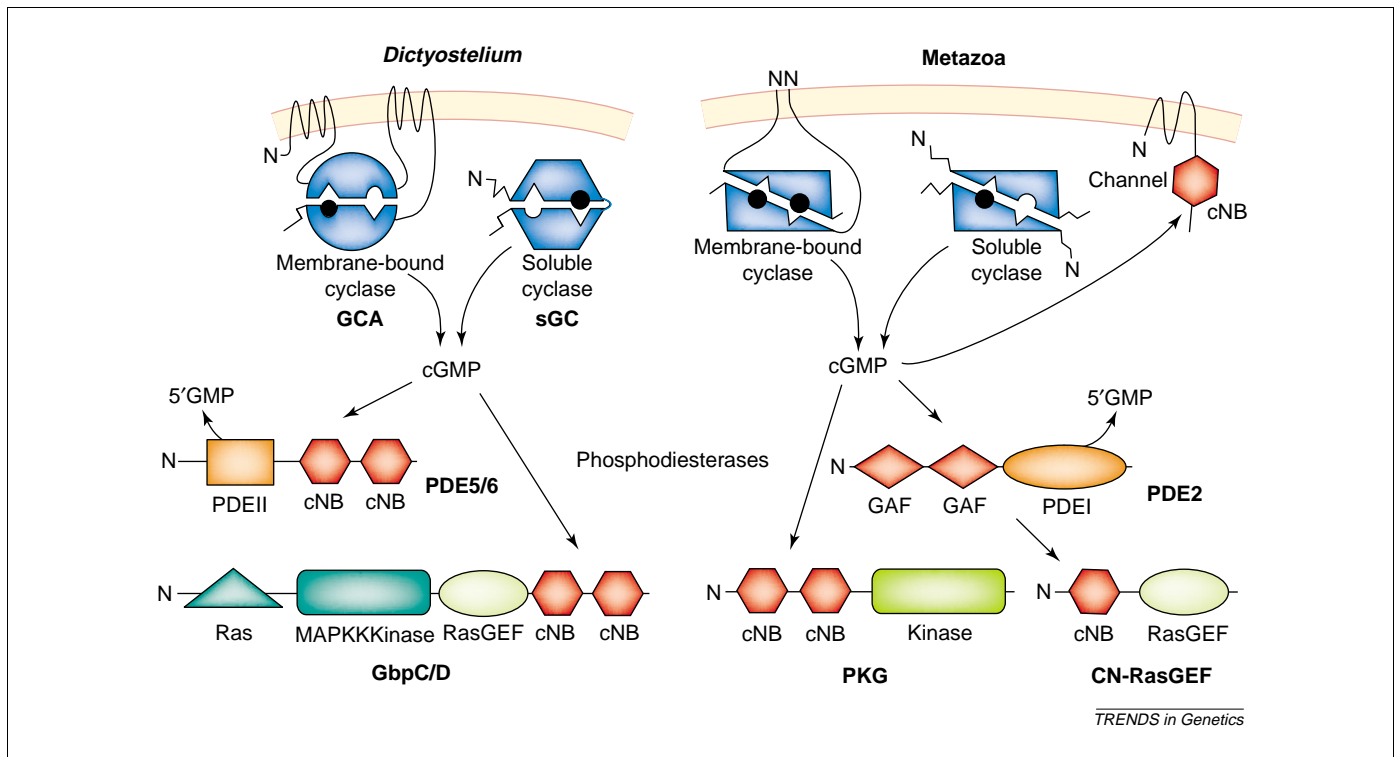


Fig. 1. cGMP signalling in *Dictyostelium* and metazoa. Domains are colour-coded by biochemical functions, and have the same shape if they are evolutionarily closely related. cGMP is produced by cyclases (blue, with a black dot for the catalytic site) and degraded by phosphodiesterases (PDE, orange). The domains that bind cGMP are red, and the domains that mediate the downstream effects of cGMP are shades of green; N refers to the N-terminus of the proteins. The order of domains in *Dictyostelium* is generally the opposite of that in metazoa. PDE5 and PDE6 are phosphodiesterases with identical domain composition, that are encoded by the *gbpA* and *gbpB* genes, respectively. GbpD is homologous to the C-terminal part of GbpC, including the GEF and cNB domains.

and metazoa, suggesting that different cGMP-signalling pathways evolved in these two lineages.

In metazoa, cGMP is synthesized by two guanylyl cyclases, one membrane-bound and the other soluble, that have a common phylogenetic predecessor (Fig. 1). Close homologues of these proteins are not found in *Dictyostelium*. Instead, the two *Dictyostelium* guanylyl

cyclases, GCA and sGC, resemble adenylyl cyclases: GCA has the topology of the ubiquitous twelve membrane-spanning metazoan adenylyl cyclases, whereas sGC is homologous to a small family of soluble adenylyl cyclases present in vertebrates and bacteria [1,2].

Cyclic GMP is degraded in metazoa by class I phosphodiesterases, several of which are activated by cGMP-binding to GAF domains [3]. *Dictyostelium* also expresses cGMP-activated phosphodiesterases (PDE5 and PDE6; original names GbpA and GbpB, respectively), but these enzymes have a common evolutionary origin that is entirely different from the origin of metazoan enzymes [4–6]. Instead of a class I phosphodiesterase they contain the unrelated class II phosphodiesterase found only in bacteria and primitive eukaryotes, and cGMP stimulation is mediated by cNB domains instead of GAF domains, two structurally and evolutionarily unrelated cyclic nucleotide binding domains.

In metazoa, cGMP signalling is mediated by cGMP-regulated protein kinase (PKG), ion channels and possibly Ras guanine nucleotide exchange factors (CNRasGEF), all of which possess cNB domains [7,8]. cGMP signalling in *Dictyostelium* is mediated by GbpC and D [4,5]. Although the combination of cNB and RasGEF domains in GbpC and D is reminiscent of CNRasGEF, the domains are shuffled, and in phylogenetic analysis the cNB domains from CNRasGEF group with those in protein kinases, whereas the cNB domains of GbpC and D are more closely related to bacterial CAP. The protein kinase domain of GbpC is homologous to mitogen-activated protein

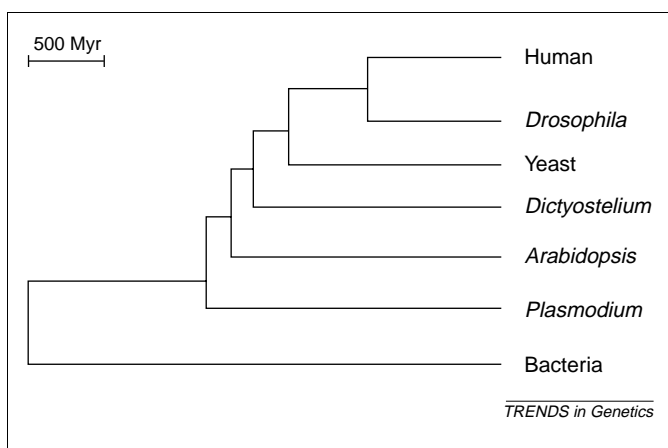


Fig. 2. Species tree. The phylogeny of species derived from the estimated divergence time of several proteins in human, *Drosophila*, yeast, plants, *Plasmodium* and bacteria [11]. The phylogenetic position of *Dictyostelium* has been determined in two independent studies [12,13]. *Dictyostelium* is a member of a group of early eukaryotes including *Entamoeba* and *Mastigamoeba*, called Canosa [13,14]. The presence of cGMP signalling in the other Canosa species is unknown. cGMP signalling is absent in yeast, plants and most bacteria, but is present in *Plasmodium* and the related species *Paramecium* and *Tetrahymena*. The scale indicates the estimated species divergence time before present. Myr, million years.

kinase kinase kinase (MAPKKK), placing it in an entirely different branch of the protein kinase superfamily from PKG. The Ras and MAPKKK region of GbpC is similar to the human protein KIAA1790, suggesting that GbpC might have arisen by fusion of a KIAA1790-like protein with a protein containing RasGEF and cNB domains.

Cyclic GMP signalling does not appear to occur in eubacteria, yeast and plants, whereas *Drosophila* and *Caenorhabditis elegans* have cGMP signalling proteins that are similar to those found in vertebrates. There is also evidence for guanylyl cyclases in cyanobacteria [9], *Paramecium*, *Tetrahymena* and *Plasmodium* [10]. As the last common ancestor of *Dictyostelium* and metazoa evolved after *Plasmodium* (Fig. 2), it will be interesting to see how other cGMP signalling proteins deduced from the completed genome of *Plasmodium* compare with the systems in *Dictyostelium* and metazoa.

At this juncture it is clear that phylogenetically distinct cGMP signalling components have evolved in the ancestor of *Dictyostelium* and in the ancestor of metazoa, to create two different but in many ways analogous cGMP signalling pathways.

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References

- 1 Roelofs, J. and Van Haastert, P.J.M. (2002) Characterization of two unusual guanylyl cyclases from *Dictyostelium*. *J. Biol. Chem.* 277, 9167–9174
- 2 Roelofs, J. and Van Haastert, P.J.M. (2001) Genes lost during evolution. *Nature* 411, 1013–1014
- 3 Francis, S.H. *et al.* (2001) Cyclic nucleotide phosphodiesterases: relating structure and function. *Prog. Nucleic Acid Res. Mol. Biol.* 65, 1–52
- 4 Goldberg, J.M. *et al.* (2002) Identification of four candidate cGMP targets in *Dictyostelium*. *Proc. Natl. Acad. Sci. U. S. A.* 99, 6749–6754
- 5 Bosgraaf, L. *et al.* (2002) A novel cGMP-signaling pathway mediating myosin phosphorylation and chemotaxis in *Dictyostelium*. *EMBO J.* 21, 4560–4570
- 6 Bosgraaf, L. *et al.* (2002) Identification and characterization of two unusual cGMP-stimulated phosphodiesterases in *Dictyostelium*. *Mol. Biol. Cell* 13, 3878–3889
- 7 De Rooij, J. *et al.* (1998) Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature* 396, 474–477
- 8 Pham, N. *et al.* (2000) The guanine nucleotide exchange factor CNrasGEF activates ras in response to cAMP and cGMP. *Curr. Biol.* 10, 555–558
- 9 Ochoa De Alda, J.A. and Houmard, J. (2000) Genomic survey of cAMP and cGMP signalling components in the cyanobacterium *Synechocystis* PCC 6803. *Microbiology* 146, 3183–3194
- 10 Linder, J.U. *et al.* (1999) Guanylyl cyclases with the topology of mammalian adenylyl cyclases and an N-terminal P-type ATPase-like domain in *Paramecium*, *Tetrahymena* and *Plasmodium*. *EMBO J.* 18, 4222–4232
- 11 Nei, M. *et al.* (2001) Estimation of divergence times from multiprotein sequences for a few mammalian species and several distantly related organisms. *Proc. Natl. Acad. Sci. U. S. A.* 98, 2497–2502
- 12 Baldauf, S.L. *et al.* (2000) A kingdom-level phylogeny of eukaryotes based on combined protein data. *Science* 290, 972–977
- 13 Baptiste, E. *et al.* (2002) The analysis of 100 genes supports the grouping of three highly divergent amoebae: *Dictyostelium*, *Entamoeba*, and *Mastigamoeba*. *Proc. Natl. Acad. Sci. U. S. A.* 99, 1414–1419
- 14 Stechmann, A. and Cavalier-Smith, T. (2002) Rooting the eukaryote tree by using a derived gene fusion. *Science* 297, 89–91

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